



8

SEQUENCE LISTING

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Immusol Incorporated

<120> Substantially Complete Ribozyme Libraries

<130> 016556-002910US

<140> US 10/067,956

<141> 2002-02-05

<150> US 60/093,828

<151> 1998-07-22

<150> US 09/357,741

<151> 1999-07-20

<160> 56

<170> PatentIn Ver. 2.1

<210> 1

<211> 12

<212> RNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:12 nucleotide
tetraloop sequence

<400> 1

ggacuucggu cc

12

<210> 2

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR primer P1,
3' adeno-associated virus inverted terminal repeat
(AAV-ITR) primer

<400> 2

aggaagatct tccattcgcc attcaggctg cgcaactgtt g

41

<210> 3

<211> 72

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR primer P2,
5' oligonucleotide with sequences for tRNAval
promoter and ribozyme library genes

<220>
 <221> modified_base
 <222> (1)..(72)
 <223> n = g, a, c or t

<400> 3
 ataccacaac gtgtgtttct ctggtnnnt tctnnnnnnn ggatcctgtt tccgcccggt 60
 ttcgaaccgg gg 72

<210> 4
 <211> 72
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:PCR primer P3,
 oligonucleotide containing ribozyme library gene
 complementary to P2 oligonucleotide

<220>
 <221> modified_base
 <222> (1)..(72)
 <223> n = g, a, c or t

<400> 4
 ccccggttcg aaaccgggcg gaaacaggat ccnnnnnnna gaannnnacc agagaaacac 60
 acgttggtgt at 72

<210> 5
 <211> 40
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:PCR primer P1,
 5' adeno-associated virus inverted terminal repeat
 (AAV-ITR) primer

<400> 5
 aggagatctg cggaagagcg cccaatacgc aaaccgcctc 40

<210> 6
 <211> 63
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide Oligo 1

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated g

<220>
 <221> modified_base
 <222> (2)..(63)
 <223> n = g, a, c or t

<400> 6
 natccacccc ccnnnnnnna gaannnnacc agagaaacac acgttggtgt atattacctg 60
 gta 63

<210> 7
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide Oligo 3

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated c

<400> 7
 ngggtaccag gtaatata 18

<210> 8
 <211> 101
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide Oligo 4

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated a

<220>
 <221> modified_base
 <222> (2)..(101)
 <223> n = g, a, c or t

<400> 8
 nattctgcag atatccatca cactggcggg gatcctcgag nnnnnnnnag aannnnacca 60
 gagaaacaca cggacttcgg tccgtggtat attacctggt a 101

<210> 9
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide Oligo 5

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated c

<400> 9
 ntcgaggatc cccgccagtg tgatggatat ctgcag

36

<210> 10
 <211> 48
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide Oligo 6

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated g

<400> 10
 ncgtaccagg taatatacca cggaccgaag tccgtgtgtt tctctggt

48

<210> 11
 <211> 87
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide Oligo 7

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated c

<220>
 <221> modified_base
 <222> (2)..(87)
 <223> n = g, a, c or t

<400> 11
 ngaaaccggg cggaacagg atccnnnnnn nnagaannnn accagagaga aacacacgga 60
 ctcggtccg tggtatatta cctggta 87

<210> 12
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide Oligo 8

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated g

<400> 12
 ngatcctggt tccgcccgtt tt

22

<210> 13
 <211> 48
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ligation
 oligonucleotide oligo 3

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated c

<400> 13
 ngcgtaggag gtaatatacc acggaccgaa gtcggtgtgt ttctctgg

48

<210> 14
 <211> 92
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:libbam PCR
 primer

<220>
 <221> modified_base
 <222> (1)..(92)
 <223> n = g, a, c or t

<400> 14
 ccccgggggg atccnnnnnn nnagaavnnn accagagaaa cacacggact tcggtccgtg 60
 gtatattacc tggtacgcgt ttttgcattt tt 92

<210> 15
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:EBVlibeco PCR
 primer

<400> 15
 tggggtggga gatatcgctg ttcctta

27

<210> 16
 <211> 87
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:annealing
 oligonucleotide Oligo1 (underline)

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated c

<220>
 <221> modified_base
 <222> (2)..(87)
 <223> n = g, a, c or t

<400> 16
 ngcgtaccag gtaatatacc acggaccgaa gtccgtgtgt ttctctggtn nnnttctnnn 60
 nnnnnggatc ctgtttccgc ccggttt 87

<210> 17
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:annealing
 oligonucleotide Oligo2 (underline)

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated g

<400> 17
 ntccgtggta tattacctgg ta 22

<210> 18
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:annealing
 oligonucleotide Oligo3 (underline)

<220>
 <221> modified_base
 <222> (1)
 <223> n = phosphorylated c

<400> 18
 ngaaaccggg cggaacagg 20

<210> 19
 <211> 16
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:cloned ribozyme
 sequence containing regions of ribozyme forming
 helix with target RNA

<400> 19
 aaaauuuuag aagcgg

16

<210> 20
 <211> 16
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer matching
 sense sequence

<220>
 <221> modified_base
 <222> (5)
 <223> n = g, a, c or t

<400> 20
 ccgcngtcaa aatttt

16

<210> 21
 <211> 16
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:primer matching
 antisense sequence

<220>
 <221> modified_base
 <222> (12)
 <223> n = g, a, c or t

<400> 21
 aaaattttga cngcgg

16

<210> 22
 <211> 56
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:RT-PCR polyT
 primer

<220>
 <221> modified_base
 <222> (56)
 <223> n = g, a, c or t

<400> 22
 tgttaccaat ctgaaggtgg gagctttttt tttttttttt tttttttttt ttttttn 56

<210> 23
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:second round
 RT-PCR primer

<400> 23
 tgttaccaat atgaagtggg agc 23

<210> 24
 <211> 16
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:sequence of
 ribozyme known to cleave PCNA mRNA

<400> 24
 gagcccugag aaggcg 16

<210> 25
 <211> 16
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:ribozyme
 sequence tag (RST) for ribozyme known to cleave
 PCNA mRNA

<220>
 <221> modified_base
 <222> (5)
 <223> n = g, a, c or u

<400> 25
 cgccngucca gggcuc 16

<210> 26
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR
amplification primer 5' PA flanking the ribozyme
expressing cassette

<400> 26

ccgttggttt ccgtagtgta gtgg

24

<210> 27

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCR
amplification primer 3' PA flanking the ribozyme
expressing cassette

<400> 27

gcattctagt tgtggtttgt cc

22

<210> 28

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ribozyme G1
ribozyme sequence tag (RST) sequence

<400> 28

gccangtccc gggtt

15

<210> 29

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:ribozyme G1
ribozyme sequence used to determine RST

<400> 29

aaccggaga atggc

15

<210> 30

<211> 58

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:double stranded
DNA tetraloop ribozyme gene template for PCR
mutagenesis reaction

<220>
 <221> modified_base
 <222> (1)..(58)
 <223> n = g, a, c or t

 <400> 30
 agaannnnac cagagaaaca cacggacttc ggtccgtggt atattacctg gtacgcgt 58

 <210> 31
 <211> 44
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:mutagenic
 oligonucleotide PCR mutagenesis reaction primer
 containing 5' end gene sequences including target
 recognition sequence

 <400> 31
 gatatcggat cccaacaact agaacggcac cagagaaaca cacg 44

 <210> 32
 <211> 25
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:sense RT-PCR
 primer for probe preparation

 <400> 32
 cagaagtacc tgagctcgcc agtga 25

 <210> 33
 <211> 25
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:antisense
 RT-PCR primer for probe preparation

 <400> 33
 gcaggcagtt gggcattggt gtaga 25

 <210> 34
 <211> 24
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:sense RT-PCR
 generated probe

 <400> 34
 gacccgagct ttgattgact ccgt 24

<210> 35
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:antisense
 RT-PCR generated probe

<400> 35
 ggtgggcatc tgcgctctag ga

22

<210> 36
 <211> 43
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:biotinylated
 RST primer, oligonucleotide containing RST for
 PCNA ribozyme

<220>

<221> modified_base

<222> (1)

<223> n = biotinylated g

<220>

<221> modified_base

<222> (39)

<223> n = g, a, c or t

<400> 36
 ncatgctcct ctagactcga ggaattcgag ccctggacna ggc

43

<210> 37
 <211> 16
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:PCNA RST primer

<220>

<221> modified_base

<222> (12)

<223> n = g, a, c or t

<400> 37
 gagccctgga cnaggc

16

<210> 38
 <211> 45
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:double stranded
 DNA adaptor

<400> 38
 gctacagctc tccggatcca agcttgatca tgacgtaatt ctgag 45

<210> 39
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial
 Sequence:adaptor-specific primer

<400> 39
 agctctccgg atccaagctt gatc 24

<210> 40
 <211> 38
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:PCR
 amplification polyG primer

<220>
 <221> modified_base
 <222> (25)..(36)
 <223> n = i

<220>
 <221> modified_base
 <222> (38)
 <223> n = g, a, c or t

<400> 40
 gaagaattct cgagggggccg cgggnngggn ngggnnngn 38

<210> 41
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:PCR
 amplification Tag-Specific Primer

<400> 41
 gaagaattct cgagggggccg c 21

<210> 42
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:GUC hairpin
 ribozyme-encoding gene subsequence

<220>
 <221> modified_base
 <222> (1)..(18)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (1)..(4)
 <223> n = g, a, c or t, n at positions 1-4 may be
 present or absent

<400> 42
 nnnnnnnnnn agaannnn

18

<210> 43
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:degenerate
 primer based on known RSTs

<220>
 <221> modified_base
 <222> (1)..(19)
 <223> n = g, a, c or t

<400> 43
 rrrrngtcr rrrrrnnnn

19

<210> 44
 <211> 15
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:(GGGII)-3
 Primer

<220>
 <221> modified_base
 <222> (1)..(15)
 <223> n = i

<400> 44
 gggnggggnn ggggn

15

<210> 45
 <211> 46
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:hairpin
 ribozyme

<220>
 <221> modified_base
 <222> (1)..(46)
 <223> n = g, a, c or u

<400> 45
 nnnagaannn naccagagaa cacacguugu gguauauuac cuggua

46

<210> 46
 <211> 11
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-cleaved
 auto-catalytic ribozyme sequence

<220>
 <221> modified_base
 <222> (1)..(11)
 <223> n = g, a, c or u

<400> 46
 uacccccnnb n

11

<210> 47
 <211> 15
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-cleaved
 auto-catalytic ribozyme sequence

<220>
 <221> modified_base
 <222> (1)..(15)
 <223> n = g, a, c or u

<400> 47
 nnnnnnnaga avnnn

15

<210> 48
 <211> 15
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:portion of
 charged ribozyme ligated to cleavage product

<220>
 <221> modified_base
 <222> (1)..(15)
 <223> n = g, a, c or u

<400> 48
 nnnbngucnn nnnnn

15

<210> 49
 <211> 21
 <212> RNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:trans-ligated
 ribozyme, target specific ribozyme

<220>
 <221> modified_base
 <222> (1)..(21)
 <223> n = g, a, c or u

<400> 49
 uacccccnnb ngucnnnnnn n

21

<210> 50
 <211> 16
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:P3 ribozyme
 sequence

<400> 50
 nnnnnnnntc ttnnnn

16

<210> 51
 <211> 16
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:P2 ribozyme
 sequence

<400> 51
 nnnnaagann nnnnnn

16

<210> 52
 <211> 16
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:P2 + P3
 ribozyme cloning sequence

<400> 52
nnnnttctnn nnnnnn

16

<210> 53
<211> 71
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:5' SL-1
auxiliary sequence

<400> 53
gcacuauggg cgcagcguca augacgcuga cgguacaggc cagacaauua gugucuuggu 60
auagugcgag g 71

<210> 54
<211> 12
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:5' tetra-loop
auxiliary sequence

<400> 54
ggacaauggu cc 12

<210> 55
<211> 101
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribozyme
containing 5' tetra-loop, 3' tetra loop and
auto-catalytic sequence

<400> 55
ggacaauggu cccacgacac aacaagaagg caaccagaga aacacacguu gugguauauu 60
accugguacg cguccuggga acaggugccc gucuguugug u 101

<210> 56
<211> 160
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:ribozyme
containing 5' SL-1, 3' tetra-loop and
auto-catalytic sequence

<400> 56
gcacuauggg cgcagcguca augacgcuga cgguacaggc cagacaauua gugucuuggu 60
auagugcgag gcacgacaca acaagaaggc aaccagagaa acacacguug ugguauauua 120
ccugguacgc guccugggaa caggugcccg ucuguugugu 160